Metabolomic and Non-Steroidal Anti-Inflammatory Drugs

Subjects: Others
Contributor: SOUMITA GHOSH

Non-steroidal anti-inflammatory drugs (NSAIDs) are Food and Drug Administration (FDA) approved antipyretic, anti-inflammatory, and analgesic drugs to mitigate pain, however it is associated with gastrointestinal injury and cardiovascular disease in some individuals. Metabolomics allows the use of biological samples to identify useful pathways involved in disease progression, and subsequently inform a greater understanding of the disease pathogenesis.

Keywords: metabolomics ; non-steroidal anti-inflammatory drugs ; pathway analysis

1. Introduction

Metabolomics is a top-down system biological tool to study metabolome. This field consists of the application of statistical tools on the data obtained by advanced analytical platforms on bio-fluids or tissues. The metabolome is the collection of endogenous small molecules like amino acids, fatty acids, breakdown products of amino acids, etc., of cellular biochemistry or that derived from host microbiota under normal or abnormal cellular processes [1]. These molecules are products of chemical reaction in a biological system. Further these molecules have widespread functions in the host like signaling, development, and physiological functions. It is thus a comprehensive study of the overall metabolism [2]. Due to its proximity to phenotype, metabolome is often used for the biomarker-determination of a disease.

NSAIDs constitute for 5% of all the drugs sold over the counter. Despite being popular drugs for the mitigation of pain and inflammation, these drugs are, however, known to cause several side effects like gastrointestinal (GI) pathology [3][4] and cardiovascular disease [5]. 40% of all the NSAIDs users have symptoms like gastroesophageal reflux and dyspeptic symptoms [6]. The onset of the symptoms depends on the nature of the NSAIDs consumed. Studies showed that Cyclooxygenase-2 (COX-2)-selective NSAIDs were associated with less symptomatic and endoscopically detected ulcers as opposed to non-selective NSAIDs like naproxen, ibuprofen etc. The symptoms were not predictive of the presence of mucosal injury. While 50% patients who exhibited symptoms had no mucosal injury, more than 50% had peptic ulcers and had no previous symptoms [7].

Some groups have demonstrated the role of arachidonic acid (AA) derivatives for the adverse cardiac functions [8][9]. Often, the drug's adverse effects like gut damages are asymptomatic and are associated with life-threatening ulceration and bleeding before they could be detected. Thus, there is an urgent need to personalize the drugs according to specific needs and susceptibility of the patients toward the side effects. Consequently, early detection of GI damage or cardiovascular disease through biomarker discovery and related pathophysiological mechanisms are critical in controlling the associated complications. Metabolic fingerprints in biofluids and tissues would help to inform practical applications as a next-generation tool for offering solutions to problems in NSAID-related side effects.

2. Metabolomics Study to Understand Metabolic Alterations by NSAIDs

2.1. Metabolomics and Metabolic Fingerprinting Techniques

Metabolome is very sensitive to internal and external stressors and thus is a probe to phenotype. The metabolic readout of the host is referred to as "metabotype" [10]. Metabolomics combined with other "omics" like proteomics; genomics can potentially elucidate the pathways involved in the process in a comprehensive way.

The detection and analyses of metabolites is complex. Various analytical platforms are used in this field [11]. There has been an immense development of the technologies used for metabolomics which has consequently led to the development of detection of the less abundant molecules reliably in biofluid mixture. Nuclear magnetic resonance (NMR), liquid chromatography (LC), gas chromatography (GC) coupled to mass spectrometry (MS) and capillary electrophoresis

(CE) have been used in metabolomics for the purpose of metabolite quantification [12] as well as structural characterization [13]. Each technique has its own advantages and disadvantages. NMR is widely used in metabolomics for reproducibility, ease in sample handling and non-destructive nature of the sample assessment. Further, only one internal standard is enough for the quantitation of all the molecules present in the sample which makes this technique cost effective. NMR in metabolomic experiments rely on ^{1}H NMR spectroscopy, as all the metabolites hold ^{1}H nucleus. For the metabolomics study, most extensively used pulse sequence is the first transient of the NOESY (Nuclear Overhauser Effect spectroscopy). The pulse sequence is (RD-90°-τ-90°- τmix-90°-ACQ), where RD = interscan relaxation delay, τ = short delay, and τmix = mixing time. This pulse sequence is abundantly used for urine and in tissue extracts free of proteins. This pulse program provides good water suppression and produce reproducible results [14].

In serum and plasma, presence of proteins and other large molecules causes a significant challenge to observe the metabolite signals. In such cases, specific pulse sequences such as CPMG (Curr-Purcell-Meiboom-Gill) are used, that attenuate the broad signals from the macromolecules [15]. The detection of metabolite signal by higher dimensionality NMR like 2D COSY, TOCSY has significantly improved the quantitation of overlapped signal in the spectra [16]. Contrary to NMR, mass spectrometry (MS) technique is used to detect low abundant molecules in the system [17][18]. It is popular for its high sensitivity and high throughput nature. However, since it uses ionization technique, the sample is often lost in the analysis. Nevertheless, since very small volume of samples are injected, the loss is often irrelevant to the user. The MS is often coupled with (LC), (GC), or (CE). LC requires the use of columns to separate the compounds based on the polarities. The most used ionization in LC is electrospray ionization (ESI). ESI being soft ionization technique facilitates the determination of molecular mass of parent ion. While Triple Quad and Ion trap are often used as mass analyzers for the targeted metabolomics, TOF analyzers are used for untargeted metabolomics. Following the spectra acquisition, the analysis follows a pipeline. NMR spectra is generated with signature from several compounds. Thus, a database containing the spectral profiles of pure compounds is matched to the mixtures of the biofluid to get specific information [19] [20]. Further, untargeted metabolomics involves the workflow with global profiling, identification of peaks in spectra, and quantitation of the peaks. In targeted profiling, the area under the peak is normalized to peak area of the internal standard under consideration for quantitation. Following peak quantitation in all platforms, the data are analyzed using different multivariate tools like principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) [21].

Metabolomics addressed various biological questions over the decades, like studying global effects of genetic manipulations [22], diseases [23][24][25], disease prediction [26]; the review discusses all the studies that have used metabolomics to explore the possible metabolic effects due to administration of nonsteroidal anti-inflammatory drugs (NSAIDs). The review primarily discusses the studies to enhance our understanding on the metabolic effects of NSAIDs by analyzing the pathways involved in various studies. The data in each paper are used to perform “pathway analysis” which is further studied in detail to see if common pathways are affected in each of those studies.

2.2. Study Design of the Metabolomics Experiment in NSAIDs Based Toxicology Study

Like other clinical and pre-clinical investigations, metabolomics studies should have a well-defined question to address. The design must clearly include subjects or model organisms that have received certain drugs and that there should exist a placebo/control group. A well-planned study that aims to examine NSAIDs response should have multiple doses and multiple time points for sample collection in humans. However, acute GI injury can be invoked by a single dose of NSAIDs like indomethacin, ibuprofenprofen, and naproxen at the doses of 25 mg/kg, 800 mg/kg, and 100 mg/kg respectively when administered orally to rats. It is relevant to explore the metabolic profile in biofluids and tissue samples at different time points post drug administration as that would shed light on the metabolic response in context of drug concentration in host. Cardiovascular effects are induced by chronic administration of COX-2 selective inhibitors like rofecoxib at 50 mg/L for 3 months in mice. Moreover, including different types of NSIADs will further incorporate the specificity of the result toward a certain type of NSAIDs. In order to explore biomarkers specific to gut damage, biofluids such as urine or serum are required to be collected for exploration. However, to understand the mechanistic aspect of the GI lesion or cardiovascular disease, specific tissues should be collected for metabolite profiling which will provide understanding of the disease pathophysiology.

Exposure to NSAIDs causes a change in the gene expressions [27], protein expression [28], and further metabolite level change in the host [29]. This discipline focusses on understanding the effects of NSAIDs on small molecule component of biofluids. Most important and relevant question in the field is to understand the molecular signatures due the metabolic perturbations by NSAIDs which will further lead to biomarker discovery and related mechanistic details.

2.3. Sample Preparations and Storage for Metabolomics Experiment

For metabolomics studies, appropriate sample storage procedures should ensure that all samples are treated in the same manner. The first step in this field is to identify the biofluid that would answer the specific biological question at hand. Sample handling is very important in this field as metabolites are affected by mishandling of the samples and poor storage conditions [30]. It is thus crucial to snap freeze the samples immediately after dissection to minimize factors such as, freeze-thaw cycles and contamination and to reduce small changes to the metabolic profiles. Freezing immediately is important as some metabolites degrade very rapidly if enzymatic activity is not stopped completely. Thus, the tissues should be frozen at –80 °C till the extraction as that preserves the integrity of the metabolome [31]. Sample preparation is also a time-consuming as well as error-prone bio-analytical step, particularly when handling complex biological matrices such as blood fluids [32]. Consequently, no universal technique suitable for blood fluid sample preparation for metabolomic fingerprinting exists [33]. Urine should be collected in azide solution to minimize bacterial growth [34]. Any bacterial growth in urine can potentially change the molecules of interest. Biological samples for metabolomics analyses require a standardized extraction protocol in laboratory settings with associated techniques available. For targeted assay, the protocol should be validated for measurement of metabolites under considerations. Pooled quality control, blanks must be included with each run to monitor the variability in extractions, acquisitions etc. Depending on tissue/biofluid under consideration, and the biological question asked, the analytical platform should be fixed, and the samples must be processed.

3. Metabolomics of Murine Response toward NSAIDs Administration

Although NSAIDs are used worldwide as anti-inflammatory drugs, it is not without side effects. One of the failures in this front of eradicating the side effects of the drugs is due to the lack of proper understanding of drug–host interaction. While it is challenging to study the side effects of drugs in humans, murine subjects provide a suitable alternative. This is because murine subjects belong to homogenous genetic background, and they thrive in controlled conditions in laboratory. These models offer the perfect opportunity to follow the metabolic effects in different treatments with parallel monitoring of their histological changes. Drug–host interaction is very similar in humans as in rats like GI lesions and myocardial infraction. Theoretically, the biomarkers obtained in the rodents should have a translational value, however before using in clinical settings the biomarkers should be validated in humans.

Nine studies (2011–2021) have been performed in the front of metabolomics and NSAIDs. Three of the studies used targeted lipidomics approach [8][35][36] while rest six studies used the approach of untargeted metabolomics [37][38][39][40][41][42]. All studies involved murine subjects. Most studies are centered around the biofluid explorations like urine and serum for biomarker discovery. Further, two studies used stomach tissue to understand the molecular level perturbations in stomach tissues by NSAIDs administration. Overall, the studies show metabolic reprogramming due to NSAIDs administration and there are common pathways affected in different studies.

4. Future Research

The future studies should be thus directed toward understanding the perturbations in the TCA cycle, amino acid, as well as fatty acid metabolism by NSAIDs administration. Importantly, the role of endoplasmic reticulum and mitochondria in alterations of the pathway requires further study.

Further studies can explore the mechanism by which TCA is dysregulated by NSAIDs and possible involvement of other organs such as gut, liver, heart, and kidney to shed light on the molecular defects in the pathogenesis of GI. To understand the therapeutic targets of the TCA cycle intermediates, interventions at the tissue level are warranted as urine analysis does not allow predicting the actual target organ where one should use the drug. This further requires many tissues to be harvested from mice administered with NSAIDs. Furthermore, diverse analytical platforms need to be involved to cancel bias, to understand the mechanistic aspects involved in the TCA cycle. A further insight into the specific enzymes in the pathway will delineate the exact nature of perturbations in the NSAIDs administration and shed light on the mechanistic aspect.

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